

The relationship between serum C-reactive protein to albumin ratio and the severity and prognosis of COVID-19

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ABSTRACT

Aims: During the coronavirus disease 2019 (COVID-19) pandemic, early identification of especially severe and critical cases may improve prognosis. In this context, laboratory parameters play an important role. This study investigated the relationship between the serum C-reactive protein (CRP)/albumin ratio (CAR) and disease prognosis in patients with COVID-19.

Methods: This study included patients who were hospitalized with a possible or confirmed diagnosis of COVID-19 at the Chest Diseases Clinic of the University of Health Sciences Dışkapı Yıldırım Beyazıt Training and Research Hospital Health Practice and Research Center between March 1, 2020, and March 31, 2021. Data on the patients were retrospectively reviewed using the hospital's record system. A total of 444 patients were included in the study and divided into two groups: those with a diagnosis of severe disease (n=141) and those without (n=303). The serum CAR was compared between the two groups. In addition, the relationship between CAR and demographic, clinical, and laboratory findings was evaluated.

Results: The mean CAR in the group with severe disease was found to be 36.1, while it was 17.1 in the group without severe disease, and the difference was statistically significant ($p<0.001$). ROC analyses showed that CAR had higher area under the curve (AUC) values compared to CRP alone in predicting severe disease, the development of complications, need for respiratory support or intensive care, and mortality. The optimal threshold values identified for CAR were as follows: 26.06 for predicting severe disease (sensitivity 66%, specificity 64%), 26.04 for complications (sensitivity 61%, specificity 62%), 26.06 for intensive care need (sensitivity 63%, specificity 62%), 25.21 for need for respiratory support (sensitivity 60%, specificity 60%), and 27.2 for mortality (sensitivity 62%, specificity 59%). These findings demonstrate that an increased CAR level is significantly associated with poor prognosis in COVID-19 patients. The relationship between serum CAR and the severity of COVID-19.

Conclusion: The data obtained indicate that the CAR is a potential biomarker for predicting severe disease and poor prognosis in COVID-19 patients. Therefore, integrating CAR into the clinical evaluation process may contribute to the early identification of high-risk patients and the timely implementation of appropriate treatment approaches.

Keywords: SARS-CoV-2, CAR, C-reactive protein, albumin

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in Wuhan, China in December 2019, rapidly spread globally and led to the Coronavirus Disease 2019 (COVID-19) pandemic. The World Health Organization (WHO) declared it an international public health emergency, and the number of cases and deaths increased rapidly. While the disease can present with mild symptoms, severe and critical conditions develop in some cases, increasing the risk of mortality. Early identification of individuals with severe illness can enable timely treatment planning and potentially improve clinical outcomes. Therefore, reliable biomarkers that can predict disease severity are needed.

The hyperinflammatory response observed in COVID-19 can be evaluated using certain serum biomarkers. Among these, the C-reactive protein (CRP) to albumin ratio (CAR), which reflects both inflammation and nutritional status, has drawn attention. Previous studies have demonstrated that elevated CAR is associated with poor prognosis and increased mortality in various clinical conditions, including infections and malignancies. For instance, in patients with sepsis, CAR has been reported to be an effective predictor of mortality.¹ Similarly, a study investigating the role of CAR in predicting mortality among patients with acute pancreatitis demonstrated that it could serve as a prognostic

factor.² Furthermore, elevated CAR levels have been associated with higher mortality in malignancies such as small-cell lung cancer, non-small-cell lung cancer, and lung adenocarcinoma.³⁻⁵ In a study conducted by Kim et al.,⁶ CAR was found to be a more sensitive prognostic indicator than CRP or albumin alone. Moreover, an investigation carried out in an intensive care unit (ICU) reported that CAR was a clinically relevant prognostic marker for predicting 28-day mortality.⁷ In conclusion, these findings support the potential utility of CAR as a prognostic indicator in critically ill patients. This study aims to evaluate the relationship between CAR at the time of admission and the development of severe disease and poor prognosis in patients hospitalized with a diagnosis of COVID-19.

METHODS

Ethics

This study was approved by the Clinical Research Ethics Committee of the University of Health Sciences (UHS) Dışkapı Yıldırım Beyazıt Training and Research Hospital (Date: 21.09.2020, Decision No: 96/11). The research process was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

This retrospective study includes 444 patients who were hospitalized with a diagnosis of COVID-19 between March 1, 2020, and March 31, 2021, at the Department of Pulmonary Diseases, University of Health Sciences (UHS) Dışkapı Yıldırım Beyazıt Training and Research Hospital Health Practice and Research Center. Demographic, clinical, radiological, and laboratory data of the patients; treatment approaches used; complications that developed; need for respiratory support and intensive care; and hospital outcomes (discharge/death) were retrospectively evaluated using the hospital information management system.

Measurement of Biochemical Parameters

CRP levels were measured using the immunoturbidimetric method with the Siemens ADVIA Chemistry XPT automated biochemistry analyzer (Siemens Healthineers, Germany) from venous blood samples taken at the time of admission. The results were recorded in milligrams per liter (mg/L).

Serum albumin levels were determined from the same samples using the bromocresol green (BCG) method, with the same analyzer. Measurements were recorded in grams per deciliter (g/dl). The CAR was calculated for each patient using their CRP and albumin values.

Determination of Disease Severity

Patients were grouped according to the WHO classification of COVID-19 disease severity. Critical illness was defined by the presence of life-supporting conditions such as acute respiratory distress syndrome (ARDS), sepsis, septic shock, the need for invasive or non-invasive mechanical ventilation (NIMV), or vasopressor therapy. Severe disease was characterized by oxygen saturation (SpO₂) below 90%, a respiratory rate greater than 30 breaths per minute, or signs of significant respiratory distress (use of accessory respiratory muscles, inability to complete sentences while speaking). Patients who did not meet any of these criteria were considered to have non-severe disease. Those who met

the criteria for critical illness were included in the severe group, while those who did not were classified in the non-severe group.

Inclusion Criteria

Patients with the following characteristics were included in the study:

- Patients aged 18 years and older
- Those who met the definition of a “suspected case” according to the guideline published by the Turkish Ministry of Health, General Directorate of Public Health during the pandemic period
- Patients who tested positive for SARS-CoV-2 by molecular diagnostic method (reverse transcription-polymerase chain reaction)
- Patients who had findings consistent with COVID-19 pneumonia on chest computed tomography (e.g., bilateral ground-glass opacities, subpleural consolidation) and were started on treatment according to the national guideline

Exclusion Criteria

Before inclusion in the study, patients with the following characteristics were excluded from evaluation:

- Pregnant individuals
- Those with another active infectious focus unrelated to COVID-19 (e.g., urinary tract infection, bacterial pneumonia, etc.)
- Patients with a diagnosis of rheumatologic or autoimmune diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis, vasculitis)
- Patients with a diagnosis of malignancy (cancer) that could affect serum albumin levels
- Individuals with chronic liver disease (e.g., cirrhosis, chronic hepatitis)
- Patients assessed to have clinical or biochemical malnutrition
- Individuals with a body-mass index ≥ 40 kg/m², consistent with morbid obesity

Statistical Analysis

The data analysis was performed using SPSS version 21.0 (IBM®, Chicago, USA). The normality of distribution of variables was assessed using visual methods (histogram and probability plots) and analytical methods (Shapiro-Wilk test). Descriptive statistics were presented as mean $\pm$ standard deviation for normally distributed numerical data, median and minimum–maximum range for non-normally distributed numerical data, and as number and percentage for nominal variables. Normally distributed numerical variables were analyzed between two groups using the Independent Samples T-test, while non-normally distributed numerical variables were analyzed using the Mann-Whitney U test. Nominal data

were evaluated between two groups using the Pearson Chi-square test or Fisher's Exact test. To determine the factors affecting COVID-19 severity, complication development, need for ICU and respiratory support, and mortality, the area under the curve (AUC) in receiver operating characteristic (ROC) analysis was evaluated, and the data were expressed with a 95% confidence interval. In the statistical analyses of this study, a p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 444 patients were included in the study. According to the WHO classification of COVID-19 disease severity, 141 patients (31.8%) were classified in the severe disease group, while 303 patients (68.2%) were in the non-severe disease group. Individuals in the severe disease group were significantly older compared to those in the non-severe group ($p=0.001$), and symptoms such as dyspnea ($p=0.044$) and loss of appetite ($p=0.014$) were observed more frequently in this group. Additionally, in patients with severe COVID-19, body temperature was higher ($p<0.001$), diastolic blood pressure was lower ($p=0.006$), SpO_2 was lower ($p<0.001$), and respiratory rate was higher ($p<0.001$). All these findings indicate significant associations between severe disease and certain clinical and symptomatic characteristics, as shown in **Table 1**.

Table 1. Comparison of clinical and demographic characteristics between severe and non-severe COVID-19 patients

Characteristics	Non-severe COVID-19 (n=303)	Severe COVID-19 (n=141)	p value
Age (median, years)	64	70	0.001
Female sex, n (%)	144 (47.5)	64 (45.4)	0.675
Symptoms			
Dyspnea, n (%)	218 (71.9)	114 (80.9)	0.044
Loss of appetite, n (%)	105 (34.7)	66 (46.8)	0.014
Comorbidities			
Hypertension, n (%)	158 (52.1)	82 (58.2)	0.237
Diabetes mellitus, n (%)	101 (33.3)	55 (39.0)	0.244
Clinical findings			
Body temperature ($^{\circ}C$, median)	36.7	37.0	<0.001
Diastolic blood pressure (mmHg, median)	75	70	0.006
Oxygen saturation (% median)	88	75	<0.001
Respiratory rate (median/minute)	22	26	<0.001

COVID 19: Coronavirus disease 2019

The vast majority of the 444 patients included in the study (84.5%) received oxygen therapy during hospitalization. ICU support was required in 31.1% of the patients. Among those who needed respiratory support, 27.5% received NIMV, 13.3% received invasive mechanical ventilation (IMV), and 28% were treated with high-flow nasal oxygen (HFNO). The overall mortality rate was found to be 11.8% ($n=52$). These findings indicate that the need for advanced respiratory support and the risk of mortality are considerable in COVID-19 patients.

It was determined that many laboratory parameters significantly differed between severe and non-severe COVID-19 patients (**Table 2**). In the severe group, leukocyte, neutrophil, CRP, ferritin, D-dimer, and pro-B natriuretic peptide (Pro-BNP) levels were significantly higher ($p<0.001$), while lymphocyte and albumin levels were significantly lower ($p<0.001$). Additionally, the CAR was significantly higher in the severe disease group ($p<0.001$). The neutrophil-to-lymphocyte ratio (NLR) was also markedly elevated in severe cases ($p<0.001$). These findings indicate that systemic inflammation and tissue damage are more pronounced in severe COVID-19 cases.

Table 2. Selected laboratory parameters in severe and non-severe COVID-19 patients

Parameter	Non severe COVID-19 (n=303)	Severe COVID-19 (n=141)	p value
Leukocyte ($\times 10^9/L$)	5.9	7.7	<0.001
Lymphocyte ($\times 10^9/L$)	0.8	0.5	<0.001
Neutrophil ($\times 10^9/L$)	4.6	6.3	<0.001
NLR	5.3	10.5	<0.001
CRP (mg/L)	62	116	<0.001
Albumin (g/dl)	3.5	3.2	<0.001
CAR	17.1	36.1	<0.001
Ferritin (ng/ml)	390.5	835.5	<0.001
D-dimer ($\mu g/ml$)	1.0	2.7	<0.001
Pro-BNP (pg/ml)	379.5	1297	<0.001

COVID 19: Coronavirus disease 2019, NLR: Neutrophil/lymphocyte ratio, CRP: C-reactive protein, CAR: CRP/albumin ratio, Pro-BNP: Pro-B-type natriuretic peptide

Based on the ROC analysis, D-dimer level showed the highest diagnostic accuracy in predicting severe COVID-19, with an AUC value of 0.759. The CAR was also considered a significant marker for predicting disease severity, with an AUC of 0.694. Ferritin and CRP levels similarly stood out with high AUC values (AUC: 0.688 and 0.681, respectively). On the other hand, lymphocyte count showed a significant inverse association, with an AUC of 0.316, indicating that lower lymphocyte levels may be associated with severe disease (**Table 3**).

Table 3. AUC values of predictive parameters for severe COVID-19 disease

Parameter	AUC	%95 GA	p value
D-dimer ($\mu g/ml$)	0.759	0.714–0.805	<0.001
CRP/albumin ratio (CAR)	0.694	0.642–0.746	<0.001
Ferritin (ng/ml)	0.688	0.634–0.741	<0.001
C-reactive protein (CRP) (mg/L)	0.681	0.629–0.733	<0.001
Lymphocyte ($\times 10^9/L$)	0.316	0.263–0.368	<0.001

AUC: Area under the curve, COVID 19: Coronavirus disease 2019

The ROC analysis for the biomarkers effective in predicting severe disease is presented in **Figure**.

In the prediction of disease severity in COVID-19, the optimal cut-off value of the CAR was determined to be 26.06 (with 66% sensitivity and 64% specificity); for the prediction

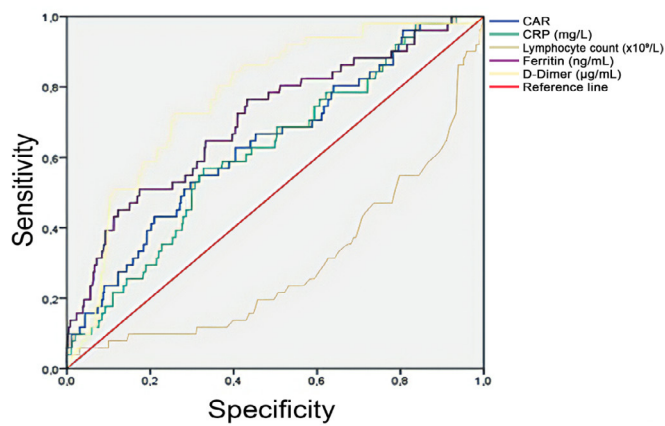


Figure. The role of predictive markers in estimating severe disease in COVID-19
COVID 19: Coronavirus disease 2019, CRP: C-reactive protein

of complications, 26.04 (61% sensitivity, 62% specificity); for the prediction of ICU requirement, 26.06 (63% sensitivity, 62% specificity); for the prediction of the need for respiratory support, 25.21 (60% sensitivity, 60% specificity); and for the prediction of mortality, 27.2 (62% sensitivity, 59% specificity).

In non-severe COVID-19 patients, CAR showed a statistically significant positive correlation with age, body temperature, respiratory rate, leukocyte and neutrophil counts, neutrophil/lymphocyte ratio (NLR), ferritin, D-dimer, interleukin-6 (IL-6), and CRP levels; and a negative correlation with SpO_2 , lymphocyte count, and albumin levels ($p < 0.05$).

The correlation results are presented in **Table 4**.

DISCUSSION

In this study, the relationship between the CAR and disease severity was evaluated in patients with COVID-19. The findings revealed that CAR levels were significantly higher in individuals in the severe disease group. Severe disease was also characterized by more advanced clinical features, such as older age, increased inflammatory response, and pronounced respiratory impairment. In this group, biomarkers reflecting

inflammation and tissue damage—such as CRP, ferritin, D-dimer, and Pro-BNP—were significantly elevated, while lymphocyte and albumin levels were notably decreased. This indicates that systemic inflammation increases with disease severity and that the immune response may be suppressed. CAR, in particular, may serve as a more comprehensive biomarker for predicting disease severity, as it reflects both elevated CRP and decreased albumin levels. The ROC analysis demonstrated that CAR has significant diagnostic value in predicting severe COVID-19 disease. Furthermore, correlation analyses showed that CAR was significantly associated with many clinical and laboratory parameters, especially in the non-severe patient group. In this group, CAR had a positive correlation with age, respiratory rate, body temperature, leukocyte and neutrophil counts, ferritin, and D-dimer levels, and a negative correlation with lymphocyte and albumin levels, as well as SpO_2 . These findings suggest that CAR may be a sensitive indicator of inflammatory activity, particularly in the early stages of the disease. In the severe patient group, however, these correlations weakened or lost statistical significance. This may indicate that the inflammatory response becomes more complex in the later stages of the disease and that CAR plays a more prominent prognostic role in the early phase. In conclusion, CAR is a biomarker that may contribute to clinical decision-making by reflecting both systemic inflammation and disease severity. However, to increase the generalizability of these findings and to clearly define its clinical applications, multicenter, prospective studies with larger sample sizes are needed.

The clinical course of COVID-19 infection varies depending on individual factors and the intensity of the immune response. Advanced age is a significant risk factor associated with more severe forms of the disease. This relationship is reflected in higher hospitalization rates, increased need for intensive care, and elevated mortality.⁸⁻¹⁰ In more severe forms of the infection, in addition to prominent respiratory symptoms such as fever, hypoxia, and tachypnea, clinical manifestations like hemodynamic instability are more frequently observed. In our current study, individuals diagnosed with severe disease were shown to be significantly

Table 4. Correlation of CRP/albumin ratio with clinical and laboratory parameters

Parameter	Non-severe COVID-19 (n=303)	p value	Severe COVID-19 (n=141)	p value
Age	$\rho=0.210$	<0.001	$\rho=-0.082$	0.337
Body temperature (°C)	$\rho=0.139$	0.015	$\rho=0.039$	0.649
Oxygen saturation (%)	$\rho=-0.459$	<0.001	$\rho=-0.112$	0.186
Respiratory rate (/min)	$\rho=0.306$	<0.001	$\rho=0.026$	0.761
Diastolic blood pressure (mmHg)	$\rho=0.144$	0.012	$\rho=-0.019$	0.823
Leukocyte ($\times 10^9/L$)	$\rho=0.265$	<0.001	$\rho=0.200$	0.017
Neutrophil ($\times 10^9/L$)	$\rho=0.377$	<0.001	$\rho=0.181$	0.032
Lymphocyte ($\times 10^9/L$)	$\rho=-0.292$	<0.001	$\rho=-0.019$	0.827
Neutrophil/lymphocyte ratio (NLR)	$\rho=0.478$	<0.001	$\rho=0.182$	0.030
Albumin (g/dl)	$\rho=-0.480$	<0.001	$\rho=-0.392$	<0.001
Ferritin (ng/ml)	$\rho=0.416$	<0.001	$\rho=0.143$	0.092
D-Dimer ($\mu g/ml$)	$\rho=0.376$	<0.001	$\rho=0.152$	0.072
C-reactive protein (CRP, mg/L)	$\rho=0.986$	<0.001	$\rho=0.973$	<0.001

COVID 19: Coronavirus disease 2019

older. In this patient group, symptoms and findings such as dyspnea, loss of appetite, elevated body temperature, hypoxia, tachypnea, and decreased diastolic blood pressure were more frequently observed. In line with other studies, severe COVID-19 patients also showed multiple laboratory abnormalities, including low albumin and lymphocyte levels, and elevated leukocyte, neutrophil, D-dimer, CRP, and ferritin levels.^{11,12} In this study as well, the laboratory findings of patients with severe disease were consistent with previous reports. Moreover, the ROC analysis results demonstrated that these parameters are statistically significant markers for predicting disease prognosis and mortality.

CRP and albumin are laboratory parameters that are commonly used in clinical practice due to their high accessibility, low cost, and rapid turnaround time. CRP, an acute-phase reactant, reflects the intensity of the inflammatory process, while albumin levels provide insight into a patient's nutritional status and overall systemic response. Elevated CRP levels are associated with poor prognosis in conditions such as infection, sepsis, tissue damage, and malignancies, whereas low albumin levels are considered an independent predictor of mortality. The combination of these two parameters—CAR—offers the advantage of simultaneously assessing both the level of inflammation and the patient's metabolic state, potentially making it a stronger prognostic marker than using either biomarker alone.⁶ The CAR has been evaluated as a prognostic indicator not only in infectious diseases but also in a wide range of clinical conditions. Particularly in conditions involving systemic inflammatory responses—such as sepsis, acute pancreatitis, and malignancy—CAR has been shown to be associated with mortality.¹⁻⁶ In a study by Fairclough et al.¹³ on acute medical admissions, patients with CAR > 2 had a significantly higher mortality rate. Similarly, in a study of intensive care patients, CAR was found to predict 28-day mortality with 64.2% sensitivity and 52.7% specificity, with a calculated cut-off value of 34.3.⁷ Another study in septic patients found that CAR measured at admission and discharge was effective in predicting 90-day mortality, with an AUC of 0.612 for admission CAR and 0.619 for discharge CAR.¹

Additionally, Kim et al.⁶ reported that CAR was superior to CRP in predicting mortality among patients with sepsis and septic shock, and that CAR values above 5.09 were significantly associated with 180-day mortality. The prognostic value of CAR has also been emphasized in cancer patients. For example, Zhou et al.³ found that individuals with small cell lung cancer who had a pre-treatment CAR > 0.441 had worse survival outcomes. In stage 4 non-small cell lung cancer patients receiving palliative chemotherapy, CAR was found to be a prognostic factor for overall survival, and in the adenocarcinoma subgroup, a cut-off value of 0.195 was determined to be significant.⁴ Another study in patients undergoing surgery for stage 3A lung adenocarcinoma reported that CAR, with a cut-off value of 0.6, was a significant prognostic factor for recurrence-free survival.⁵

Among the biomarkers used to evaluate systemic inflammation in COVID-19 patients, CRP and albumin levels measured at hospital admission offer practical advantages due to their rapid turnaround, low cost, and widespread availability. Accumulating evidence supporting

the prognostic value of these parameters in various clinical conditions has also been extended to the context of COVID-19, where similar associations are being explored. In COVID-19, CRP levels are known to rise in response to an excessive cytokine release and inflammatory reaction, and this increase has been shown to correlate with disease severity, mortality, radiological severity, and respiratory failure.¹⁴⁻¹⁹ On the other hand, low serum albumin levels have also been reported to be associated with poor clinical outcomes.^{20,21}

Recent studies have proposed that the CAR may serve as an effective biomarker for predicting disease severity and mortality in COVID-19 patients.²²⁻²⁴ In the literature, studies examining the prognostic value of CAR in COVID-19 patients have reported varying threshold values and predictive strengths. For example, a retrospective study involving 90 patients identified a positive correlation between CAR and disease severity, and determined a cut-off value of 0.296 with 76.7% sensitivity and 80.4% specificity for distinguishing severe disease.²² In another study evaluating 197 patients, the AUC value of CAR measured at admission for predicting severe disease was calculated as 0.718, with a cut-off of 0.9 yielding 69.1% sensitivity and 70.8% specificity.²³ In a study conducted by Günay et al.,²⁴ patients were divided into three groups based on CAR levels, and in-hospital mortality was found to be 8.2 times higher in the group with the highest CAR levels compared to the lowest group. The findings in our study are consistent with this literature. CAR levels were found to be statistically significantly higher in the severe disease group. ROC analysis demonstrated the diagnostic power of CAR in predicting adverse outcomes such as severe disease, development of complications, intensive care requirement, need for respiratory support, and mortality, with cut-off values of 26.06; 26.04; 26.06; 25.21; and 27.2, respectively. The sensitivity for these threshold values ranged between 60–66%, and specificity between 59–64%. Additionally, the AUC value for CAR was higher than that of CRP alone, supporting the prognostic performance of the ratio. In correlation analyses, in the severe disease group, CAR showed a significant positive correlation with leukocyte count, neutrophil count, NLR, AST, ALT, CRP, ESR, and procalcitonin levels, while a negative correlation was observed with albumin levels. No significant correlations were found with other parameters. These findings suggest that CAR is a highly applicable biomarker with the potential to reflect systemic inflammation and disease severity in COVID-19 patients.

Limitations and Future Directions

This study has several limitations. Firstly, due to its retrospective design, data were obtained from existing records, which carries the risk of potential data omissions and information bias. Additionally, the study was conducted at a single center, which may limit the generalizability of the findings. Furthermore, only certain comorbidities and clinical conditions could be excluded from the study, and it was not possible to fully control for all potential confounding diseases or conditions that could affect CRP and albumin levels. This may introduce some uncertainty in the interpretation of measured CAR levels. Moreover, given the dynamic nature of the inflammatory response, the evaluation of biochemical parameters at a single time point (i.e., at admission) may be

insufficient to reflect changes over time. These limitations suggest that the results should be interpreted with caution, and highlight the need for prospective, multicenter studies with larger sample sizes.

CONCLUSION

The findings of this study demonstrate that the CAR is significantly associated with disease severity and adverse clinical outcomes in COVID-19 patients. Given that CAR reflects both the inflammatory response and nutritional status, particularly in the early stages of illness, it may serve as a valuable biomarker for prognostic assessment. Routine evaluation of CAR in clinical practice may aid in the early identification of high-risk patients and facilitate timely implementation of appropriate treatment strategies. However, to validate these findings and clearly define the role of CAR in clinical practice, further large-scale, multicenter, and prospectively designed studies are warranted.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study was approved by the Clinical Research Ethics Committee of the University of Health Sciences (UHS) Dışkapı Yıldırım Beyazıt Training and Research Hospital (Date: 21.09.2020, Decision No: 96/11).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer reviewed

Conflict of Interest Statement

The authors declare that they have no conflicts of interest to disclose.

Financial Disclosure

The authors declare that this study did not receive any financial support.

Author Contributions

All authors declare that they contributed to the design, execution, and analysis of the study and have approved the final version of the manuscript.

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